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| 13. ABSTRACT (Maximum 200 Words)<br><br>The current high dose chemotherapy for leukemia and other hematological malignancies is very toxic and therefore therapeutic targeting would be particularly useful. Phage display of random peptides or short proteins has been used in vivo to identify tissue-specific endothelial address molecules. Coupling of these homing molecules to cytotoxic drugs or therapeutic cells has resulted in targeted therapies with a low toxicity and a good efficacy profile.<br><br>We have recently characterized several bone marrow (BM) and tumor targeting peptides/proteins from phage libraries displaying 6-10 random amino acids or cDNA molecules, and by utilizing a combination of ex vivo and in vivo phage display. The main purpose of this project is to develop an efficient cancer therapy targeted to BM. As a first step, we are currently constructing drug molecules where a BM targeting peptide is coupled to nucleoside antimetabolites in order to decrease dose or usage limiting non-BM toxicity. These constructs will be tested for therapeutic and targeting efficacy on leukemic cells and in mice leukemia models. |   |  |   |   |
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## INTRODUCTION

A long-standing goal for both clinicians and researchers is the ability to target therapeutic agents to the appropriate location in the body. Targeting would be useful for high dose cancer chemotherapy, which is toxic to both normal and malignant tissues. Even patients initially cured from the disease suffer from long-term side effects of therapy, such as secondary malignancies, infertility, premature osteoporosis and chronic graft versus host disease (after allogenic stem cell transplantation).

Phage display is an efficient method to select for peptides, antibodies or recombinant proteins that specifically bind pure macromolecules, cultured cells or ex vivo cell and tissue preparations (1, 2). Phage display has also been used in vivo to identify peptides that target tissue specific endothelial address molecules (3). Our group has characterized peptides that home to the vasculature of 10 individual organs (4). Coupling of these homing peptides to cytotoxic drugs, such as doxorubicin, has resulted in a compound that is more effective and less toxic than the native untargeted drug (5, 6). Short peptides composed of two functional domains, one a tumor endothelium homing motif (NGR or RGD-4C) and the other an apoptosis-inducing sequence ([KLAKKLAK]<sub>2</sub>), have also been designed (7). These constructs had marked anti-tumor effects in a mouse model. Peptides isolated from phage display libraries can be therapeutically effective themselves, such as a CTTHWGFTLC-peptide which inhibits tumor angiogenesis in vivo by blocking matrix metalloproteinase activity (8). It seems likely that many of the problems related to therapeutic peptides (e.g. delivery, stability, synthesis) can be overcome in the near future (9). Tumor targeting could be utilized also in radioimmunotherapy, antibody mediated immunotherapy, antisense oligonucleotide therapy and most probably in cell therapy.

I have isolated peptides that home to bone marrow (BM) and propose to use them for targeted drug delivery in mice (10). As a novel approach, I have recently cloned cDNA molecules into T7 phage, which allows for the selection of natural ligands binding to receptors on endothelial cells. This is a significant advantage both in receptor isolation and in understanding the physiologic/pathologic phenomena under study as random peptides rarely are homologous to natural ligands.

## BODY

### *Description of the training*

Kimmo Porkka, M.D., Ph.D. (principal investigator) has received research training in clinical epidemiology, clinical cancer research and human molecular genetics at 2 universities in Finland. His clinical training includes specialist degrees in general internal medicine and clinical hematology at the Helsinki University Central Hospital, Finland. Subsequently, he decided to pursue basic cancer research of complement his clinical duties. Since April 1999 he has worked at the Burnham Institute on phage display screening. He will return to Finland in 1-2 years and aim to continue the current research work at the Department of Hematology, University of Helsinki along with his clinical duties. The long-term purpose of this project is to develop novel therapies that could be tested in clinical trials among patients suffering from hematological malignancies.

### *Results*

We have isolated several different peptide motifs homing to BM from phage libraries displaying 6-10 random residues. The results indicate that ex vivo prescreening on BM mononuclear cells, including BM endothelial cells, may restrict the libraries in a biologically meaningful manner and select for phage that specifically bind in vivo with good affinity to BM. We are currently constructing targeted drug molecules, where BM homing peptide motif is coupled to nucleoside antimetabolites (cytarabine, fludarabine).

Recently I have isolated several clones/natural ligands binding both to lineage depleted BM cells and tumor endothelial cells by using cDNA phage display (see Methods). The prescreening was done on lineage depleted stem cell like population and the in vivo screen on highly vascularized human AML M3 xenograft tumors. The screen capitalizes on the shared phenotype between hematopoietic/hemangiopoietic stem cells and angiogenic tumor cells: phage pool binding to BM stem cells is highly enriched on phage binding to tumor vessels. Most of these clones represent novel genes found only in large HGP high throughput genomic clones. We are currently characterizing these in more detail and aim to isolate the corresponding receptors using both

affinity chromatography and a high capacity retroviral expression system developed in our laboratory. These novel ligand-receptor pairs could be valuable both for targeting purposes and in understanding the functional characteristics of angiogenic tumor vasculature.

## KEY RESEARCH ACCOMPLISHMENTS

- Isolation of bone marrow homing peptide sequences
- Setting up a protein (cDNA) phage display system and isolating natural ligands binding to bone marrow stem cells and to tumor endothelial cells
- Setting up a tumor mouse model with subcutaneous human leukemia implants to test the efficacy and toxicity of targeted molecules
- Histological analysis tumors

## REPORTABLE OUTCOMES

### *Abstract*

- Kimmo Porkka, Pirjo Laakkonen, Daniel Rajotte, Jason Hoffman, Erkki Ruoslahti: BONE MARROW HOMING PEPTIDES FROM PHAGE DISPLAY LIBRARIES. Poster at the American Society of Hematology Meeting in New Orleans December 1999.

## CONCLUSIONS

If targeted chemotherapy with small homing peptides proves successful, it would change the current practice of treating hematological and other cancers in a very significant way. Firstly, markedly higher concentrations of the chemotherapeutic agent would be reached in the tumor resulting in an increase in dose intensity, which may translate into an increase in the cure rates. Secondly, many drugs that are too toxic when used in a systemic manner or cannot be delivered in therapeutically effective concentrations, may prove useful if the delivery is confined to the vicinity of the tumor tissue. Thirdly, compared to current regimens with severe short term (e.g. infections, mucositis, diarrhea) and long term (e.g. infertility, secondary malignancies) side effects, targeted therapy has the potential of saving normal healthy tissues from the cytotoxic effects of drugs. Lastly, BM targeting enables the testing and effective use of novel therapy forms (e.g. stem cell or cytotoxic gene therapy in vivo; proapoptotic or anti-angiogenic therapy; terminal differentiation-inducing therapy).

Here I report the success of isolating peptide/protein ligands that bind to specific receptors on endothelial cells residing in bone marrow or tumors. The short proteins derived from cDNA libraries most likely represent natural ligands that are expressed on the surface of cells and mediate their homing to specific tissues. This is an advantage over random peptides, which rarely resemble natural ligands. Next step of this project will be the isolation of the putative receptors responsible for ligand binding observed here. The identification of a natural product's receptor provides an important link between its phenotype and cellular component.

The experimental approaches for receptor isolation will include cell-based screening and affinity chromatography. I aim to utilize the recently developed display cloning techniques, which combines protein identification with gene isolation.



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## STEM OR PROGENITOR CELL BIOLOGY II

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**BONE MARROW HOMING PEPTIDES FROM PHAGE DISPLAY LIBRARIES.** Kimmo Porkka\*,<sup>1,2</sup> Pirjo Laakkonen\*,<sup>1</sup> Daniel Rajotte\*,<sup>1</sup> Jason Hoffman\*,<sup>1</sup> Erkki Ruoslahti\*.<sup>1</sup> (Intr. by Wadih Arap) <sup>1</sup>*Cancer Research Center, The Burnham Institute, La Jolla, CA;* <sup>2</sup>*Dept. Medicine, Div. Hematology, Helsinki U. Central Hospital, Helsinki, Finland.*

Phage display is an efficient method to select peptides, antibodies or recombinant proteins that specifically bind pure macromolecules, cultured cells or *ex vivo* cell and tissue preparations. Phage display has also been used *in vivo* to identify peptides that target tissue-specific endothelial address molecules. We used M13-derived FUSE5 phage libraries to isolate peptides that home to bone marrow (BM). We utilized a library that displays 6 random amino acids on the pIII coat protein and a combination of *ex vivo* and *in vivo* phage display. The *ex vivo* prescreening was performed by incubating  $10^6$  BM mononuclear cells with  $10^9$  transducing units (TU) of phage for 90 min at 4°C. After 3 rounds of selection, the inserts displayed by the individual phage were sequenced. The phage pool that resulted from the third round of *ex vivo* screening was subsequently used *in vivo*. The *in vivo* selection was performed by intravenously injecting  $10^9$  TU of the round 3, *ex vivo* screened phage into BALB/c mice. After 5 minutes of circulation, the mice were sacrificed, the BM flushed from their femurs, and washed 3 times with 1% BSA in PBS. The phage in the BM were then rescued, amplified, purified and used in an additional round of selection. Three rounds of *in vivo* selection were performed and the inserts sequenced. In a separate set of experiments, we did 8 rounds of selection exclusively *in vivo*. A total of 11 different peptide motifs appeared more than once in the various rounds of selection. When individually tested *ex vivo* and *in vivo*, 5 of these phage homed to BM more than a control, insertless fd-tet phage. *Ex vivo* and *in vivo* binding data were congruent. These phage did not bind to blood. The phage were 20-30 fold enriched in their BM homing compared to control organs kidney and brain. The phage that homed most efficiently were from the *ex vivo*-prescreened libraries; individual phage derived from *in vivo* selections alone did not show specific binding to BM. Our results indicate that *ex vivo* prescreening on BM mononuclear cells, including BM endothelial cells, may restrict the libraries in a biologically meaningful manner and select for phage that bind specifically and efficiently to BM *in vivo*. A similar strategy can be utilized to screen for phage binding to human BM. These peptide sequences may be useful for targeting therapeutic agents (drugs, cells, gene therapy vectors) to BM and for isolating ligands responsible for the homing of cells to BM.